

SYSTEM AND METHOD FOR PROVIDING OPTICAL MICROSCOPY**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] The application claims the benefit of priority of Singapore patent application No. 10202300596P, filed 6 March 2023, the content of it being hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] The disclosure relates to a system and method for providing optical microscopy, in particular, label-free far-field optical microscopy.

BACKGROUND

[0003] The following discussion of the background is intended to facilitate an understanding of the present disclosure only. It should be appreciated that the discussion is not an acknowledgement or admission that any of the material referred to was published, known or is part of the common general knowledge of the person skilled in the art in any jurisdiction as of the priority date of the disclosure.

[0004] Label-free microscopy has been commonly used for the visualization and analysis of samples without the need for exogenous labeling agents such as fluorescent dyes or stains. Far-field microscopy typically refers to a class of optical microscopy techniques where the resolution is ultimately limited by diffraction, as opposed to near-field microscopy where the resolution can surpass the diffraction limit.

[0005] There exists a need to provide a solution to overcome the aforementioned limitation associated with label-free far-field optical microscopy.

SUMMARY

[0006] A technical solution may be provided in the form of a system and method of label-free far-field optical microscopy that overcomes the conventional limit of the resolution imposed by the signal-to-noise ratio of the optical microscopy. To overcome the limitation in the dynamic range and signal-to-noise ratio of optical microscopy, a detector of the microscopy

optical system may be configured to spatially separate strong and weak optical signals. By sequentially measuring complex amplitudes of various modes of radiation (or scattering) from a sub-wavelength-sized particle (e.g. a nanoparticle), the label-free far-field optical microscopy technique that measures a set of complex coefficients of decomposed scattered light from the sub-wavelength-sized particle. By configuring or setting appropriate setting of basis far-field pattern, super-resolution images of the object under observation can be estimated or retrieved from the measured coefficients.

[0007] In one aspect, the present disclosure seeks to achieve super-resolution imaging of relatively small objects, for example, nanoparticles with finite size, that may be relatively thin. Under a light beam generated by a light source for illuminating the objects, scattered light from the objects can be considered as an equivalent source profile, and the confined source profile can be approximated with, for example, a Slepian series (where basis functions are orthogonal prolate spheroidal wave functions). As a principle of the present disclosure, a set of coefficients of infinite Slepian series can be measured with far-field light. The present disclosure further takes into account the modelling of higher order Slepian modes, which may be relatively weak.

[0008] In some embodiments, a spatial light modulator may be configured to separate weaker modes of scattering from stronger modes of scattering utilizing optical tomography for Slepian mode of radiation.

[0009] According to an aspect of the present disclosure, there is provided a label-free far-field optical microscopy system comprising: an illumination source configured to generate an incident light beam for illuminating a specimen; a sample platform arranged to support the specimen and produce total internal reflection of the incident light beam passing therethrough; an objective lens positioned to allow a plurality of optical signals from the specimen to pass therethrough; a spatial light modulator positioned along a back focal plane of the objective lens, the spatial light modulator configured to receive the plurality of optical signals, the plurality of optical signals comprising a first optical signal and a second optical signal, and generate corresponding modulated first optical signal and modulated second optical signal; and a detector configured to sequentially receive the modulated first optical signal and modulated second optical signal; wherein the detector comprises at least one processor configured to, based on a basis function of an expansion of far-field radiation pattern, process the first modulated optical signal and second modulated optical signal to determine a first complex coefficient and a second complex coefficient.

[0010] In some embodiments, the spatial light modulator is configured to generate the first modulated optical signal and the second modulated optical signal based on at least one mask, the at least one mask comprising an amplitude mask, a phase mask and/or a frequency mask.

[0011] In some embodiments, the basis function is based on a multipole series model or a Slepian prolate spheroidal series model.

[0012] In some embodiments, the basis function comprises the multipole series model, the multipole series model comprises a plurality of azimuthal orders (m), and a plurality of radial orders (l).

[0013] In some embodiments, the basis function comprises the Slepian series model. The Slepian series model may be constructed such that the associated Fourier transforms are bi-orthogonal both in the infinite Fourier domain, and the finite Fourier domain imposed by a numerical aperture of the objective lens.

[0014] In some embodiments, the Slepian series model is further configured to utilize common-path interferometry. In the common-path interferometry, a lowest-order Slepian series contribution of radiation pattern may be utilized as a reference signal, and wherein the first complex coefficient is associated with the reference signal, and the second complex coefficient is associated with a measurement signal and measured as a normalized form with respect to the first complex coefficient.

[0015] In some embodiments, the at least one mask is an amplitude mask, the amplitude mask comprising a first portion for masking the measurement signal, and a second portion for masking the reference signal.

[0016] In some embodiments, the illumination source is a laser source, and the sample platform comprises a sapphire cube, the sapphire cube configured in the total internal reflection (TIR) mode for laser illumination.

[0017] In some embodiments, the specimen is a nanoparticle, and the objective lens is positioned to allow scattered optical signals to pass therethrough.

[0018] In some embodiments, the measured values from one specific pixel (where camera sensor plane and optical axis intersect) are utilized.

[0019] In some embodiments, the at least one processor is configured to provide a correction matrix, the correction matrix associated with a measured set of coefficients and an ideal set of coefficients retrieval procedure for a single pixel system that utilizes peripheral pixels of the detector, wherein the detector is an array detector.

[0020] According to another aspect of the present disclosure, there is provided a method for providing label-free far-field optical microscopy, the method comprising: providing an illumination source to generate an incident light beam on a specimen; supporting, by a sample platform, the specimen, and producing total internal reflection of the incident light beam passing therethrough; providing an objective lens and a spatial light modulator positioned along a back focal plane of the objective lens, configuring the spatial light modulator to receive a plurality of optical signals, the plurality of optical signals comprising a first optical signal and a second optical signal, and generate corresponding modulated first and second optical signals; and providing a detector, the detector configured to sequentially receive the modulated first optical signal and modulated second optical signal; wherein the detector comprises at least one processor configured to, based on a basis function of an expansion of far-field radiation pattern, process the first modulated optical signal and second modulated optical signal to determine a first complex coefficient and a second complex coefficient.

[0021] In some embodiments, the method further comprises configuring the spatial light modulator to generate the first modulated optical signal and the second modulated optical signal based on at least one mask, the at least one mask comprising an amplitude mask, a phase mask and/or a frequency mask.

[0022] In some embodiments, the basis function is based on a multipole series model or a Slepian prolate spheroidal series model.

[0023] In some embodiments, the basis function comprises the multipole series model, the multipole series model comprises a plurality of azimuthal orders (m), and a plurality of radial orders (l).

[0024] In some embodiments, the basis function comprises the Slepian series model. The Slepian series model may be modelled such that the associated Fourier transforms are bi-orthogonal both in the infinite Fourier domain, and the finite Fourier domain imposed by a numerical aperture of the objective lens.

[0025] In some embodiments, the Slepian series model may utilize common-path interferometry. The common-path interferometry may be configured such that a lowest-order Slepian series contribution of radiation pattern is utilized as a reference signal, and wherein the first complex coefficient is associated with the reference signal, and the second complex coefficient is associated with a measurement signal and measured as a normalized form with respect to the first complex coefficient.

[0026] In some embodiments, the at least one mask is an amplitude mask, the amplitude mask comprising a first portion for masking the measurement signal, and a second portion for masking the reference signal.

[0027] In some embodiments, the illumination source is a laser source, and the sample platform comprises a sapphire cube, the sapphire cube configured in the total internal reflection (TIR) mode for laser illumination.

[0028] In some embodiments, the specimen is a nanoparticle, and the objective lens is positioned to allow scattered optical signals to pass therethrough.

[0029] In some embodiments, the method further comprises providing a correction matrix, the correction matrix associated with a measured set of coefficients and an ideal set of coefficients retrieval procedure for a single pixel system that utilizes peripheral pixels of the detector, wherein the detector is an array detector.

[0030] According to another aspect of the present disclosure there is provided a computer-readable medium comprising program instructions, which, when executed by one or more processors, cause the one or more processors to process the first modulated optical signal and second modulated optical signal according to the aforementioned method to determine a first complex coefficient and a second complex coefficient for the expansion of far-field radiation pattern.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The disclosure will be better understood, with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

- FIG. 1 shows a schematic block diagram of a system for providing optical microscopy according to various embodiments of the present disclosure;
- FIG. 2A illustrates a schematic concept of an optical system utilizing optical tomography configured in a multipole mode, based on a multipole series model, according to an embodiment of the present disclosure; FIG. 2B shows a comparison table between conventional technology and the optical system of FIG. 2A;
- FIG. 3A shows an example of a plurality of retrieved multipole coefficients based on the multipole series model;

- FIG. 3B shows a comparison of Euclidean distances between multipole coefficients from a test object and multipole coefficients from various reference objects;
- FIG. 3C illustrates a retrieved shape associated with the smallest Euclidean distance;
- FIGS. 4A to 4C illustrate another schematic concept of an optical system wherein the scattered light incident upon the illuminated specimen is modeled as an equivalent localized source profile, and the optical signals are processed based on a basis function to determine a plurality of complex coefficients;
- FIG. 5A shows an embodiment of the optical system based on the schematic concept of FIGS. 4A to 4C, utilizing a digital micromirror device (DMD) as a spatial light modulator;
- FIG. 5B shows an embodiment of measuring or determining intensities of two reference radiations and the relative phase between the two reference radiations based on a Slepian series as the basis function, utilizing common-path interferometry;
- FIG. 5C illustrates the determination of the normalized complex coefficients based on the measured values of FIG. 5B;
- FIG. 6A illustrates some examples of specimens and their retrieved super-resolution images utilizing the optical microscopy system of FIG. 5A;
- FIG. 6B illustrates a refinement or correction process of any raw complex coefficients measured in the form of a correction matrix;
- FIG. 6C illustrates a correction matrix M in the demonstration; and
- FIG. 7 illustrates a general flowchart of a method for providing optical microscopy.

DETAILED DESCRIPTION

[0032] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the disclosure may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the disclosure. Other embodiments may be utilized and structural, logical changes may be made without departing from the scope of the disclosure. The various embodiments are not necessarily mutually exclusive, as some embodiments can be combined with one or more other embodiments to form new embodiments.

[0033] Embodiments described in the context of one of the systems or methods are analogously valid for the other systems or methods.

[0034] Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments. Features that are described in the context of an embodiment may correspondingly be applicable to the other embodiments, even if not explicitly described in these other embodiments. Furthermore, additions and/or combinations and/or alternatives as described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

[0035] In the context of some embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

[0036] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0037] As used herein, the term “processor(s)” includes one or more electrical circuits capable of processing data, i.e. processing circuits. A processor may include analog circuits or components, digital circuits or components, or hybrid circuits or components. Any other kind of implementation of the respective functions which will be described in more detail below may also be understood as a “circuit” in accordance with an alternative embodiment. A digital circuit may be understood as any kind of a logic implementing entity, which may be special purpose circuitry or a processor executing software stored in a memory, or a firmware.

[0038] As used herein, the term “label-free” broadly refers to imaging or optical microscopy that allows for the visualization and analysis of samples or objects of various sizes, without the need for exogenous labeling agents such as fluorescent dyes or stains. Instead of relying on fluorescent or chemical markers to highlight specific structures or molecules within the samples, label-free microscopy techniques utilize inherent contrast mechanisms present in the sample itself, such as variations in refractive index, optical density, or phase. The aforementioned samples or objects may include biological samples and nanoparticles.

[0039] As used herein, the term “light” refers to electromagnetic radiation with varying wavelengths, and are visible to the human eye (i.e. within the visible spectrum).

[0040] As used herein, the term “far-field” microscopy broadly refers to various optical microscopy techniques where the resolution is ultimately limited by a diffraction parameter. In far-field microscopy, the resolution may be limited by the wavelength of light and a numerical aperture of the objective lens of the optical system.

[0041] As used herein, the term “back focal plane (BFP)” may refer to the Fourier plane or the conjugate plane, and broadly refers to a plane in the optical system where the Fourier transform of the complex optical field at an exit pupil of the optical system (pupil function) is formed. In some embodiments, the BFP may be located at a distance equal to the focal length of the objective lens from the objective lens itself. In some embodiments, the spatial frequencies present in the complex optical field are mapped. These spatial frequencies correspond to the various components of the image formed by the optical system. Low spatial frequencies may correspond to relatively large-scale features in the image, while high spatial frequencies may correspond to relatively finer details.

[0042] As used herein, the term “basis function” broadly refers to a component of a mathematical basis, which is a set of functions used to represent other functions through, for example, linear combinations. In some embodiments, any function within a particular function space can be expressed as a linear combination of the basis function. In the context of the present disclosure, the basis function may be, or form part of, a far-field radiation pattern expansion.

[0043] As used herein, the term “equivalent source profile” broadly refers to a simplified representation of a complex source distribution that produces the same far-field radiation pattern as the original distribution, and typically involves a smaller number of sources or a different spatial distribution of sources that approximates the behavior of the original system. The equivalent source profile may be used in scattering analysis and other electromagnetic applications to simplify calculations.

[0044] As used herein, the term “super-resolution” image refers to an image or image data that is capable of achieving spatial resolution beyond a classical diffraction limit of light, for example, going beyond half the wavelength of the light used. Super-resolution microscopy allows for the visualization of cellular structures and molecular details at nanometer-scale resolution, and may include structured illumination microscopy (SIM).

[0045] As used herein, the term “spatial mode tomography” refers to a technique used to characterize and reconstruct a spatial mode profile of an electromagnetic radiation beam (e.g. a light beam). Spatial modes broadly include various possible distributions of light intensity and phase across the cross-section of the beam. Such modes can have different shapes, such as Gaussian, Hermite-Gaussian, Laguerre-Gaussian, or other complex patterns. In some embodiments, spatial mode tomography may include measuring the intensity and phase

distribution of the light at various planes perpendicular to the propagation direction. This data is then used to reconstruct the complete spatial mode profile of the beam. The reconstruction process typically involves mathematical techniques which may include, but are not limited to, Fourier analysis, modal decomposition, or maximum likelihood estimation.

[0046] As used herein, the term “mask(s)” refers broadly to various filters such as amplitude filters, frequency filters, and/or phase filters. It is appreciable that such mask(s) may include one or more optical components such as lens. As a non-limiting example, a vortex-like mask, also known as a vortex phase mask or vortex coronagraph, is an optical component used in imaging systems to manipulate the phase of light. As another non-limiting example, a Bessel-like mask refers to a type of spatial filter used in various applications, particularly in optics and image processing. The Bessel-like mask may have similar characteristics to a Bessel function, for example, exhibiting a central peak with concentric rings of decreasing intensity. This type of mask can be used, for instance, in optical systems to achieve specific spatial filtering or in image processing to enhance certain features while suppressing others.

[0047] As used herein, the term “data” may be understood to include information in any suitable analog or digital form, for example, provided as a file, a portion of a file, a set of files, a signal or stream, a portion of a signal or stream, a set of signals or streams, and the like. The term data, however, is not limited to the aforementioned examples and may take some forms and represent any information as understood in the art.

[0048] As used herein, the term “obtain” refers to the processor which actively obtains the inputs, or passively receives inputs from one or more sensors or data source. The term obtain may also refer to a processor, which receives or obtains inputs from a communication interface, e.g. a user interface. The processor may also receive or obtain the inputs via a memory, a register, and/or an analog-to-digital port.

[0049] As used herein, the term “module” refers to, or forms part of, or include an Application Specific Integrated Circuit (ASIC); an electrical/electronic circuit; a combinational logic circuit; a field programmable gate array (FPGA); a processor (shared, dedicated, or group) that executes code; other suitable hardware components that provide the described functionality; or a combination of some or all of the above, such as in a system-on-chip. The term module may include memory (shared, dedicated, or group) that stores code executed by the processor.

[0050] According to an aspect of the present disclosure, there is provided a label-free far-field optical microscopy system comprising: an illumination source configured to generate an incident light beam for illuminating a specimen; a sample platform arranged to support the specimen and produce total internal reflection of the incident light beam passing therethrough;

[0051] an objective lens positioned to allow a plurality of optical signals from the specimen to pass therethrough; a spatial light modulator positioned along a back focal plane of the objective lens, the spatial light modulator configured to receive the plurality of optical signals, the plurality of optical signals comprising a first optical signal and a second optical signal, and generate corresponding modulated first optical signal and modulated second optical signal; and

[0052] a detector configured to sequentially receive the modulated first optical signal and modulated second optical signal; wherein the detector comprises at least one processor configured to, based on a basis function, process the first modulated optical signal and second modulated optical signal to determine a first complex coefficient and a second complex coefficient for the expansion of far-field radiation pattern.

[0053] In the following, embodiments will be described in detail.

[0054] Referring to FIG. 1, there is a label-free far-field optical microscopy system 100 comprising: an illumination source 102 configured to generate an incident light beam on a specimen 1000 for illuminating the specimen 1000; a sample platform 104 arranged to support the specimen 1000 and produce total internal reflection of the incident light beam passing therethrough; objective lens 106, and a spatial light modulator 108 positioned along a back focal plane of the objective lens 106, the spatial light modulator 108 configured to receive a plurality of optical signals, the plurality of optical signals comprising a first optical signal 110 and a second optical signal 112, and generate corresponding modulated first optical signal 114 and second optical signal 116; and at least one processor 120 configured to, based on a basis function, process the modulated first optical signal 114 and the second modulated signal 116 to determine a first complex coefficient associated with the first optical signal and the second optical signals. The at least one processor 120 may form part of a detector. The detector may include at least one image capturing device, for example, a CCD camera. In some embodiments, the detector may include photodiodes.

[0055] The first complex coefficient and the second complex coefficient may be utilised in the expansion of far-field radiation pattern, which may be subsequently used to retrieve one or more super-resolution images of the specimen 1000.

[0056] The specimen 1000 may be any particle, include sub-wavelength-sized particle including a nanoparticle, the nanoparticle may be made of various materials such as metal, organic and inorganic dielectric, biological virus, defects in a thin-film material, and so on.

[0057] In some embodiments, the spatial light modulator 108 may be configured to generate the first modulated signal 114 and the second modulated optical signal 116 based on a plurality of amplitude, phase and/or frequency masks.

[0058] FIG. 2A illustrates a schematic concept of an optical system 200 utilizing optical tomography configured in a multipole mode, based on a multipole series model, according to an embodiment of the present disclosure. The optical system 200 seeks to overcome a limitation associated with the dynamic range of the quantitative light detection system by spatially separating weaker modes of scattering from stronger modes of scattering utilizing optical tomography. A spatial light modulator 208 may be configured with suitable masks to generate modulated optical signals sequentially to be received by a detector unit 220, the detector unit 220 may include an image-capturing device such as a camera.

[0059] In operation, a subwavelength-size object 2000 (e.g. a nanoparticle) may be illuminated by a source, such as a laser beam, and the total internal reflection illumination may be achieved using a transparent platform 204 with a relatively high refractive index, such as, but not limited to, a sapphire cube.

[0060] The scattered light from the arbitrary subwavelength-size object 2000 under total internal reflection (TIR) illumination may be modelled as a converging series, i.e. a multipole expansion of multipole radiation from a plurality of, or multiple, localized sources, with various vector spherical harmonics basis. It is appreciable that for each multipole radiation, the mathematical orthogonality property of vector spherical harmonics can be optically realized by placing appropriate amplitude masks at the spatial light modulator 208 placed along the Fourier plane of the image formed by the objective lens 206.

[0061] At the image plane, i.e. where the final magnified image of the specimen 2000 is formed for observation or analysis, modulated optical signals, in the form of focused light, that corresponds to a specific complex coefficient can be spatially separated from others and filtered for each measurement, and analysis. By sequential changing of the amplitude mask on the spatial light modulator 208, each complex amplitude of the multipole coefficient can be measured one by one.

[0062] As illustrated in FIG. 2A, the changing of amplitude masks may result in different modulated optical signals being received by an image-capturing device 218 at different times, via an aperture 240. In an exemplary case, enough level of photon number from higher order (i.e. weaker) multipole radiations can be measured by increasing the exposure time of the detector 220 (i.e. camera), which resolves the aforementioned challenge. The conceptual comparison with conventional imaging technology is summarized in the table of FIG. 2B.

[0063] In some embodiments, because of the infinitesimal rotational symmetry of conventional objective lenses along the optical axis, various azimuthal orders (m) of multipole series can be well-separated by utilizing various vortex-like masks. Among the specific azimuthal order, finite series of coefficients with various radial orders (l) can also be measured over the conventional accuracy limit by utilizing various Bessel-like masks. The complex amplitudes (both magnitude and phase) can be measured with common-path interferometry realized by each mask.

[0064] In some embodiments, the first modulated optical signal and the second modulated optical signal to determine the first complex coefficient and the second complex coefficient may be mathematically expressed as Equation (1) as follows:

$$\begin{aligned} c_1 X(l_1, m_1) \\ c_2 X(l_2, m_2) \end{aligned} \quad (1)$$

Where c_1 , c_2 are the first and second complex coefficients associated with the first modulated optical signal $X(l_1, m_1)$ and the second modulated optical signal $X(l_2, m_2)$.

[0065] Referring to FIG. 2B, based on a conceptual comparison, the present disclosure is likely able to outperform conventional technology, as the proposed optical system 200 is able to take into account higher-order (weaker) multipole radiation.

[0066] FIGS. 3A to 3C illustrate exemplary use cases of the measured or determined complex coefficients. The measured complex coefficients c_1 , c_2 , etc. may be used to produce super-resolution images or image data 302, 304, 306, 308, 310.

[0067] FIG. 3A shows a plot 320 of a plurality of retrieved multipole coefficients based on the multipole series model of a reference specimen with a known shape. The complex coefficients may be plotted across a real axis (x-axis) and imaginary axis (y-axis).

[0068] An example of the super-resolution imaging procedure is shown in FIG. 3B. FIG. 3B shows a comparison of Euclidean distances between multipole coefficients obtained from a test specimen with an unknown shape and multipole coefficients measurement from various

reference specimens of known shapes. The Euclidian distance between the set of measured coefficients and a set of each reference's coefficients may be compared.

[0069] FIG. 3C illustrates a retrieved shape of the test specimen, based on identifying the reference specimen with the smallest Euclidean distance with the test specimen. As illustrated, the identified reference specimen is sample number two, corresponding to image data 304.

[0070] Although the conventional images of the various objects may be difficult to differentiate in the above examples, the applicant noted that the multipole decomposition tomography may enable reliable shape retrieval for the dielectric sub-wavelength-size particles with feature size between $\lambda/2$ to $\lambda/10$, reproducibly, wherein λ denotes the wavelength. Based on the principle that the multipole expansion is generally applicable (i.e. generalizable) to localized source distributions, the proposed scheme can be applied to super-resolution imaging of arbitrary-shape subwavelength-size particles. FIG. 4A illustrates a schematic concept of another optical system 400 of the present disclosure. The optical system 400 comprises a sapphire cube 404, the sapphire cube 404 configured to support a nanoparticle 4000. Scattered electromagnetic radiation (light) from the nanoparticle 4000 may undergo further modulation by a spatial light modulator and be detected and processed by a processor based on the principles and models.

[0071] Based on the general principle that arbitrarily fine image resolution with label-free far-field-based optical microscopy is possible with appropriate prior knowledge of the specimen under observation, the reconstruction of super-resolution image of nanoparticles with finite size using total-internal reflection illumination based on the sapphire cube 404 may be achieved.

[0072] In some embodiments, the set-up of the optical system 400 may be configured such that the maximum lateral size of the nanoparticles may be set as 0.8λ (λ : free-space wavelength of the illuminated and scattered light). In the processing of the modulated optical signals including the first modulated optical signal and second modulated optical signal, it may be appreciable that the maximum lateral size of the nanoparticles can be adjusted or defined. In some embodiments, a problem may be defined as reconstructing a confined two-dimensional (thin in z -axis) equivalent source profile, see FIG. 4B, from its radiation profile/pattern, as shown in FIG. 4C. The confined two-dimensional arbitrary source profile can be expanded using a Slepian series (where basis functions are orthogonal prolate spheroidal wave functions)

representing the equivalent source profile as a sum of weighted orthogonal basis functions, mathematically expressed in Equation (2) as follows.

$$f(x, y) = \sum c_{N,n} \psi_{N,n}(x, y), \quad (r = \sqrt{x^2 + y^2} \leq 0.8\lambda) \quad (2)$$

Where $f(x, y)$ denotes the equivalent source profile, $\psi_{N,n}(x, y)$ is an orthogonal basis function representative of prolate spheroidal wavefunctions with radial order n and azimuthal order N , and the condition $(r = \sqrt{x^2 + y^2} \leq 0.8\lambda)$ denotes that the equivalent source profile is defined within a circle of radius 0.8λ , where λ denotes the wavelength of the free-space wavelength of the illuminated and scattered light. It may be appreciable that the 0.8 parameter may be adjusted up to 1000.

[0073] In some embodiments, the quantitative measurement or determination of the complex coefficients, $c_{N,n}$, may be determined based on analysing radiation pattern in the far-field (equivalently back focal plane of objective lens), see further details with reference to FIGS. 5A to 5C. With the measured set of coefficient and Equation (2), super-resolution images of the nanoparticles 4000 can be obtained. The achievability of the super-resolution may be originated from the prior knowledge, maximum extent of the nanoparticle (i.e. 0.8λ). This allows the approximation of source profile with Slepian series where all coefficients of basis functions can be measurable in far-field. As larger extent are allowed, eigenvalue of higher order of the Slepian series may become smaller, so achieving same degree of super-resolution becomes more challenging.

[0074] FIG. 5A shows a detailed set up of an optical system 500 based on similar concept as system 400, for the determination of the complex coefficients. A sapphire cube 504, or any other suitable platform that provides total internal reflection, may be configured for a specimen 5000, e.g. one or more nanoparticles, to rest thereon. When illuminated, the scattered light incident on the objective lens 506 may be converged and incident on a spatial light modulator, which may be in the form of a digital micromirror device (DMD) 508. The DMD 508 may be placed at the back focal plane of the objective lens 506. Coherent summation of pixel-wise multiplication of radiation pattern and a mask may be performed by lens assembly 507, and the value can be measured or determined with reference from a centre pixel of the detector 520. FIG. 5A also shows a converging/diverging lens assembly 509 positioned between the objective lens 506 and DMD 508.

[0075] FIG. 5B and FIG. 5C illustrates how various parameters associated with the complex coefficients $c_{N,n}$, and/or the normalized coefficient $c_{i,j}/c_{0,0}$, may be determined. A Slepian series $\psi_{N,n}(x, y)$ may be constructed in accordance with Equation (2), such that their Fourier transforms are bi-orthogonal both in the infinite Fourier domain, $-\infty < k_{x,y} < \infty$, and the finite Fourier domain imposed by a numerical aperture (NA) of the objective lens 506. In some embodiments, NA may be set at 0.9, $-0.9k_0 < k_{x,y} < 0.9k_0$. By using second orthogonality, each coefficient can be retrieved according to the function mathematically expressed in Equation (3) as follows,

$$\int_{-0.9k_0}^{0.9k_0} \tilde{f}(k_x, k_y) \tilde{\psi}_{i,j}^*(k_x, k_y) = \int_{-0.9k_0}^{0.9k_0} \sum c_{N,n} \tilde{\psi}_{N,n}(k_x, k_y) \tilde{\psi}_{i,j}^*(k_x, k_y) = \sqrt{\lambda_{i,j}} c_{i,j} \quad (3)$$

where $\lambda_{i,j}$ is eigenvalue of a prolate spheroidal wave function order of (i, j) . The first term can be realized optically using spatial light modulator (SLM) that realizes $\tilde{\psi}_{i,j}^*(k_x, k_y)$ in back focal plane of the objective lens (i.e., a Mask) and coherent summation by conventional lens. The measured value from the centre pixel of an image capturing device (e.g. camera) that on the optical axis corresponds to the coherent summation realized by the lens. By changing mask pattern with different (i, j) for each sequential measurement, a set of coefficients, $c_{i,j}$, can be measured. This technique may be akin to spatial mode tomography.

[0076] A specific procedure for measuring each complex coefficient $c_{i,j}$ is illustrated with reference to FIG. 5B and FIG. 5C. Common-path interferometry is utilized where reference signal and measurement signal share an optical path without using any additional light beam path. In some embodiments, the lowest-order Slepian series contribution of radiation pattern may be utilized as a reference signal. Then, all other coefficients are measured as a normalized form with reference to the complex coefficient associated with the reference signal $c_{0,0}$, i.e. $c_{i,j}/c_{0,0}$. It may be appreciable that the utilization of common-path interferometry may be more stable under mechanical vibration.

[0077] In some embodiments, before the measurement of each complex coefficient, an intensity of reference A (reference only at left part of the back focal plane), I_{R_A} , an intensity of reference B (reference only at right part of the back focal plane), I_{R_B} , and relative phase between reference A and B, $e^{i\phi_{BA}}$, are measured (see Fig. 5B). In some embodiments, the

amplitude masks used may be divided two parts, a first part for filtering or masking the measurement signal, and a second part for filtering or masking the reference signal, each measured coefficient may correspond to a specific basis function at entire back focal plane relative to reference basis function at entire back focal plane by a two-step interferogram measurement (total eight interferogram per each coefficient). Then, a total of eight intensities from each interferogram — four corresponds to left-reference signal and right-measurement signal, rest of four corresponds to left-measurement signal and right-reference signal — may be measured and used to retrieve normalized coefficient according to the mathematical equation (4) as follows:

$$\frac{c_{l,j}}{c_{0,0}} = \Sigma \left[\frac{(-i)^n I_{niS_B,RA}}{\sqrt{I_{RA}}} + e^{i\phi_{BA}} \frac{(-i)^n I_{niS_A,RB}}{\sqrt{I_{RB}}} \right] \quad (4)$$

[0078] With a relatively strong electromagnetic radiation used for illumination, the reference optical signal (i.e. lowest order Slepian series contribution) may be strong enough to be a shot-noise limited measurement system (i.e. shot noise of the reference light may be much stronger than detector noise).

[0079] In some embodiments, exposure time for the capturing of each image frame may be adjusted such that number of photoelectrons in a utilized pixel is around 1000 for the reference light. In this case, read noise may be around 1 electron, dark current may be around 0.01 – 0.001 electrons (with an exposure time of around 10 milliseconds (ms) to 100 ms, and 0.1 electron/pixel/second is the camera specification). In some embodiments, microscope drift may be actively compensated by using piezo stage and maximum likelihood estimation of three-dimensional position of nanoparticles. Therefore, the measurement system can be considered as a shot-noise-limited system. In this situation, longer measurements or integrating more frames for weaker signals that corresponds to higher order Slepian series may be adopted until good enough signal-to-noise ratio is achieved. A super-resolution image of the nanoparticle can be reconstructed using Equation (2) with measured set of coefficients from Equation (4).

[0080] FIG. 6A shows experimental demonstrations compared with conventional Fourier imaging. The first row shows scanning electron microscopy (SEM) images 601 to 605 of fabricated nanoparticles used in the experimental demonstration. The nanoparticles may be fabricated using a focused electron beam induced deposition (FEED) of platinum on a

sapphire cube. The sapphire cube may be coated with 2 nm-thick Niobium-Titanium nitride (NbTiN) to facilitate electric conduction. After platinum deposition, the NbTiN may be etched using reactive ion etching using oxygen. The estimated thickness of nanoparticles is around 30 nm. The second row 610 shows respective calculated Fourier imaging in ideal situations (i.e., no error) with a numerical aperture of 1 ($NA = 1$). A wavelength of 638 nm is assumed. The images in the second row 610 is based on the application of a Fourier filter to the original design of nanoparticles, and it may be confirmed that no nanoparticles are well-imaged. The third row 620 and the corresponding images shows similar Fourier imaging but with $NA = 3$. The fourth row 630 shows calculated Slepian imaging based on Equation (2) with total internal reflection illumination in an ideal situation (i.e., no error). To do this calculation, first, near-field data from FDTD simulation may be recorded and then transformed to far-field radiation. Next, the entire optical system may be modelled, and far-field data within the spatial frequency band limited by the numerical aperture of the objective lens, $0.9k_0$ is processed. Images shown in the fourth row 630 may be incoherent summations of images of respective nanoparticles with four different illumination directions. A total of 19 coefficients may be utilized for an image of a nanoparticle with a specific illumination direction. The fifth row 640 shows experimental Slepian imaging. The experimental results of fifth row 640 is found to show similar or better resolution compared with ideal Slepian imaging in the fourth row 630 which shows comparable performance of Fourier imaging with $NA = 3$, which is the state-of-the-art performance in label-free far-field imaging.

[0081] In some embodiments, refinement of any raw coefficients measured may be required to mitigate inaccuracies arising from distortion of set-up or other reasons. For example, experimentally measured raw coefficients may be inaccurate due to the distortion of the optical setup originating from many reasons, for example, miss-alignment, wavefront errors of optical components, dust particles on optical elements, and so on. Therefore, correction of experimental results is important to obtain accurate images.

[0082] A correction process of the 19 coefficients obtained under FIG. 6A may be described with reference to FIG. 6B. In some embodiments, the correction process may be provided in the form of a correction matrix, i.e. the at least one processor may be configured to provide a correction matrix, the correction matrix relates to a measured set of coefficients and an ideal set of coefficients retrieval procedure for a single pixel system that utilizes peripheral pixels of the array detector used in the measurement setup, for example the set up shown in FIG. 5A.

[0083] In the experimental demonstration, 19 coefficients are utilized and the Slepian series with 19 coefficients is assumed to well-approximate the objects under observation. Therefore, for linear and time-invariant systems, a corrected set of coefficients and a raw set of coefficients can be related using a 19-by-19 correction matrix, M mathematically expressed as Equation (5), as follows.

$$\begin{pmatrix} c_1 \\ \vdots \\ c_{19} \end{pmatrix}_{\text{Ideal}} = M \begin{pmatrix} c_1 \\ \vdots \\ c_{19} \end{pmatrix}_{\text{Meas}} \quad (5)$$

[0084] In order to retrieve the correction matrix, M , at least 19 independent samples were measured where each sample provides 19 coefficients so that all unknown 19-by-19 elements of M can be retrieved. Instead of measuring many samples, one specific calibration sample may be measured based on, for example, image 601, in the form of a small dot. The measured sets of coefficients may then be utilized from the peripheral pixels in addition to the centre pixel which is the sole pixel utilized for actual measurement, wherein the proposed technique can be categorized as a single pixel imaging. Measured values from different pixels correspond to Slepian expansion coefficients with different origins, so they can be considered as different samples. In some embodiments, sets of coefficients from 175 pixels within the region of interest of the basis functions of the Slepian series were utilized as shown in FIG. 6B.

[0085] An equation relating sets of coefficients from calculation and sets of coefficients from measurement for calibration sample, may be mathematically expressed as Equation (6), as follows:

$$\begin{aligned} C_{\text{Ideal}} &= M C_{\text{Meas}} \\ \text{where } C_{\text{Ideal}} &= \begin{pmatrix} c_1^{(1)} & c_1^{(2)} & \dots & c_1^{(175)} \\ \vdots & \vdots & & \vdots \\ c_{19}^{(1)} & c_{19}^{(2)} & & c_{19}^{(175)} \end{pmatrix}_{\text{Ideal}}, \\ C_{\text{Meas}} &= \begin{pmatrix} c_1^{(1)} & c_1^{(2)} & \dots & c_1^{(175)} \\ \vdots & \vdots & & \vdots \\ c_{19}^{(1)} & c_{19}^{(2)} & & c_{19}^{(175)} \end{pmatrix}_{\text{Meas}}. \end{aligned} \quad (6)$$

[0086] Then, M may be found as follows,

$$[0087] \quad M = C_{\text{Ideal}} C_{\text{Meas}}^\dagger$$

[0088] where the dagger symbol $\dagger$ indicates the Moore-Penrose inverse. FIG. 6C illustrates the retrieved correction matrix $\mathbf{M}$ in the demonstration.

[0089] According to another aspect of the disclosure, there is a method 700 for providing label-free far-field optical microscopy. The method 700 may be implemented for the device 100 in the system 200 as described.

[0090] The method 700 may comprise the following steps:

[0091] 702: providing an illumination source to generate an incident light beam on a specimen;

[0092] 704: supporting, by a sample platform, the specimen, and producing total internal reflection of the incident light beam passing therethrough;

[0093] 706: providing an objective lens and a spatial light modulator positioned along a back focal plane of the objective lens,

[0094] 708: configuring the spatial light modulator to receive a plurality of optical signals, the plurality of optical signals comprising a first optical signal and a second optical signal, and generate corresponding modulated first and second optical signals; and

[0095] 710: providing a detector, the detector configured to sequentially receive the modulated first optical signal and modulated second optical signal; wherein the detector comprises at least one processor configured to, based on a basis function, process the first modulated optical signal and second modulated optical signal to determine a first complex coefficient and a second complex coefficient for the expansion of far-field radiation pattern.

[0096] In some embodiments, the method 700 may be implemented in a computer-readable medium comprising program instructions, which, when executed by one or more processors, cause the one or more processors to perform the method 700. In some embodiments, the method 700 may be coded as software codes for execution by the processor 120.

[0097] It may be appreciable that various embodiments of the present disclosure utilizes multipole and/or Slepian series to expand radiation (or scattering) patterns and induced source configuration of sub-wavelength particles with arbitrary shapes. This may be because multipole and/or Slepian series rapidly converge for localized sources, so test particles with various shapes can be effectively described by fewer coefficients. Although various embodiments describe the modelling and utilizing of multipole series, and Slepian prolate spheroidal series for the determination of complex coefficients and subsequent generation of super-resolution images, it may be appreciated that pixel-by-pixel successive detection can also be considered for the determination of complex coefficients.

[0098] In some embodiments, the additional optical components such as lenses, mirrors, or additional spatial light modulators (SLMs) may be added as desired to manipulate the spatial properties.

[0099] In some embodiments, multiple detectors may be positioned or placed at different distances from the illumination source 102.

[00100] In some embodiments, the processing of modulated optical signals may involve applying one or more calibration procedures, background subtraction, noise reduction, and other data processing techniques.

[00101] In some embodiments, the reconstruction of a spatial mode profile may include one or more additional mathematical algorithms, such as Fourier transform-based methods, modal decomposition techniques, or iterative optimization algorithms.

[00102] While the disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the disclosure as defined by the appended claims. The scope of the disclosure is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

1. A label-free far-field optical microscopy system comprising:
 - an illumination source configured to generate an incident light beam for illuminating a specimen;
 - a sample platform arranged to support the specimen and produce total internal reflection of the incident light beam passing therethrough;
 - an objective lens positioned to allow a plurality of optical signals from the specimen to pass therethrough;
 - a spatial light modulator positioned along a back focal plane of the objective lens, the spatial light modulator configured to receive the plurality of optical signals, the plurality of optical signals comprising a first optical signal and a second optical signal, and generate corresponding modulated first optical signal and modulated second optical signal; and
 - a detector configured to sequentially receive the modulated first optical signal and modulated second optical signal;

wherein the detector comprises at least one processor configured to, based on a basis function of an expansion of far-field radiation pattern, process the first modulated optical signal and second modulated optical signal to determine a first complex coefficient and a second complex coefficient.
2. The system according to claim 1, wherein the spatial light modulator is configured to generate the first modulated optical signal and the second modulated optical signal based on at least one mask, the at least one mask comprising an amplitude mask, a phase mask and/or a frequency mask.
3. The system according to claim 1 or 2, wherein the basis function is based on a multipole series model or a Slepian prolate spheroidal series model.
4. The system according to claim 3, wherein the basis function comprises the multipole series model, the multipole series model comprises a plurality of azimuthal orders (m), and a plurality of radial orders (l).

5. The system according to claim 3, wherein the basis function comprises the Slepian series model.
6. The system according to claim 5, wherein the Slepian series model constructed such that the associated Fourier transforms are bi-orthogonal both in the infinite Fourier domain, and the finite Fourier domain imposed by a numerical aperture of the objective lens.
7. The system according to claim 6, wherein the Slepian series model is further configured to utilize common-path interferometry.
8. The system according to claim 7, wherein in the common-path interferometry, a lowest-order Slepian series contribution of radiation pattern is utilized as a reference signal, and wherein the first complex coefficient is associated with the reference signal, and the second complex coefficient is associated with a measurement signal and measured as a normalized form with respect to the first complex coefficient.
9. The system according to claim 2 and claim 8, wherein the at least one mask is an amplitude mask, the amplitude mask comprising a first portion for masking the measurement signal, and a second portion for masking the reference signal.
10. The system according to any one of the preceding claims, wherein the illumination source is a laser source, and the sample platform comprises a sapphire cube, the sapphire cube configured in the total internal reflection (TIR) mode for laser illumination.
11. The system according to any one of the preceding claims, wherein the specimen is a nanoparticle, and the objective lens is positioned to allow scattered optical signals to pass therethrough.
12. The system according to any one of the preceding claims, wherein measured values from one specific pixel (where camera sensor plane and optical axis intersect) are utilized.
13. The system according to any one of the preceding claims, wherein the at least one processor is configured to provide a correction matrix, the correction matrix associated with a measured set of coefficients and an ideal set of coefficients retrieval procedure for a single

pixel system that utilizes peripheral pixels of the detector, wherein the detector is an array detector.

14. A method for providing label-free far-field optical microscopy, the method comprising:
providing an illumination source to generate an incident light beam on a specimen;
supporting, by a sample platform, the specimen, and producing total internal reflection of the incident light beam passing therethrough;
providing an objective lens and a spatial light modulator positioned along a back focal plane of the objective lens,
configuring the spatial light modulator to receive a plurality of optical signals, the plurality of optical signals comprising a first optical signal and a second optical signal, and generate corresponding modulated first and second optical signals; and
providing a detector, the detector configured to sequentially receive the modulated first optical signal and modulated second optical signal;

wherein the detector comprises at least one processor configured to, based on a basis function of an expansion of far-field radiation pattern, process the first modulated optical signal and second modulated optical signal to determine a first complex coefficient and a second complex coefficient.

15. The method according to claim 14, further comprising configuring the spatial light modulator to generate the first modulated optical signal and the second modulated optical signal based on at least one mask, the at least one mask comprising an amplitude mask, a phase mask and/or a frequency mask.

16. The method according to claim 14 or 15, wherein the basis function is based on a multipole series model or a Slepian prolate spheroidal series model.

17. The method according to claim 16, wherein the basis function comprises the multipole series model, the multipole series model comprises a plurality of azimuthal orders (m), and a plurality of radial orders (l).

18. The method according to claim 16, wherein the basis function comprises the Slepian series model.

19. The method according to claim 18, further comprises constructing the Slepian series model such that the associated Fourier transforms are bi-orthogonal both in the infinite Fourier domain, and the finite Fourier domain imposed by a numerical aperture of the objective lens.
20. The method according to claim 19, further comprises configuring the Slepian series model to utilize common-path interferometry.
21. The method according to claim 20, wherein in the common-path interferometry configuration, a lowest-order Slepian series contribution of radiation pattern is utilized as a reference signal, and wherein the first complex coefficient is associated with the reference signal, and the second complex coefficient is associated with a measurement signal and measured as a normalized form with respect to the first complex coefficient.
22. The method according to claim 15 and claim 21, wherein the at least one mask is an amplitude mask, the amplitude mask comprising a first portion for masking the measurement signal, and a second portion for masking the reference signal.
23. The method according to any one of claims 14 to 20, wherein the illumination source is a laser source, and the sample platform comprises a sapphire cube, the sapphire cube configured in the total internal reflection (TIR) mode for laser illumination.
24. The method according to any one of claims 14 to 23, wherein the specimen is a nanoparticle, and the objective lens is positioned to allow scattered optical signals to pass therethrough.
25. The method according to any one of claims 14 to 24, further comprising providing a correction matrix, the correction matrix associated with a measured set of coefficients and an ideal set of coefficients retrieval procedure for a single pixel system that utilizes peripheral pixels of the detector, wherein the detector is an array detector.
26. A computer-readable medium comprising program instructions, which, when executed by one or more processors, cause the one or more processors to process the first modulated optical signal and second modulated optical signal according to the method of any one of claims 14 to 25, to determine a first complex coefficient and a second complex coefficient for the expansion of far-field radiation pattern.